Quercus trungkhanhensis (Fagaceae), a New Species from Cao Vit Gibbon Conservation Area, Cao Bang Province, northeastern Vietnam

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A new species, *Quercus trungkhanhensis* Binh & Ngoc (Fagaceae), from Cao Vit Gibbon Conservation Area, northeastern Vietnam is described. It is morphologically similar to *Q. engleriana* Seemen and *Q. marlipoensis* Hu & Cheng in having scaly cupules and in the shape, texture and glabrescence of the leaves, but distinguished from the former particularly by the size and morphology of the fruits (acorns and cupules) and the latter by smaller leaves with fewer lateral veins. *Quercus trungkhanhensis* is also similar to *Q. franchetii* Skan in fruit morphology, but differs in having glabrescent leaves.

Key words: Cao Vit Gibbon Conservation Area, Fagaceae, new species, Quercus, Vietnam

Quercus Linnaeus (1753) (Fagaceae), with 400-500 species (Nixon 1993, Valencia-A et al. 2016), is one of the largest genera in the Fagaceae. The genus is characterized by pendulous staminate inflorescences, carpellate flowers always solitary, capitate or dilated stigma and indehiscent cupules (Huang et al. 1999, Phengklai 2008). Some species are often dominant in various forest types, including temperate deciduous forests in eastern North America, Europe and Asia, Mediterranean and desert scrub forest in Europe, Mexico and adjacent regions, and tropical montane forests in Southeast Asia (Nixon 1993, Hubert et al. 2014, Valencia-A et al. 2016). Besides their economic and ecological importance, species of *Quercus* are also considered in many countries as cultural and patrimonial resources (Hubert et al. 2014).

Vietnam is known for the high diversity of species of Fagaceae; 216 species in 6 genera, including 44 species of *Quercus* have been record-

ed (Ho 2003, Ban 2005, Li et al. 2016, Ngoc et al. 2016). Recently, taxonomic studies of Fagaceae in Vietnam have been undertaken by Deng et al. (2010), Linh et al. (2013), Vuong and Xia (2014), Li et al. (2016) and Ngoc et al. (2016), but the taxonomy of *Quercus* remains to be revised.

Here, we report a new species of *Quercus* from Cao Vit Gibbon Conservation Area, located at Trung Khanh District, Cao Bang Province, northeastern Vietnam (Fig. 1). The conservation area was established in 2007 to strengthen conservation for the Cao Vit gibbon (*Nomascus nasutus*) and covers 7,600 ha of limestone ground where five types of vegetation are found (Tu *et al.* 2009): subtropical evergreen broad leaved forests in valleys, subtropical bamboo forests in valleys, limestone subtropical evergreen mixed forests, tropical evergreen shrub savannahs and tropical secondary evergreen grasslands. Within the conservation area, 960 species of vascular plants, of which 34 species are listed as threatened in Viet-

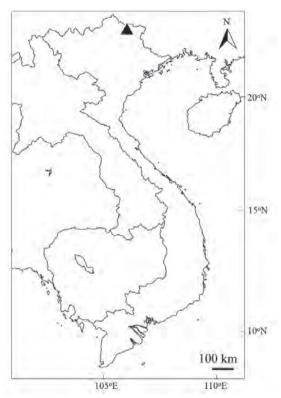


FIG. 1. Distribution map of *Quercus trungkhanhensis* Binh & Ngoc. Black triangle: Cao Vit Gibbon Area, Trung Khanh District, Cao Bang Province.

nam's Red Data Book (Ban et al. 2007, Tu et al. 2009), belonging to 144 families have been recorded.

During a botanical survey at Cao Vit Gibbon Conservation Area in 2016, we discovered an undescribed species of *Quercus*, based on morphological comparison with morphologically similar species, which we describe and illustrate below as *Quercus trungkhanhensis* Binh & Ngoc. We also provide DNA sequences of the two regions of ITS and matK for DNA barcoding, and assess its conservation status using IUCN Red List criteria (IUCN 2001).

Material and Methods

Morphological observations

To verify the validity of the new species, we thoroughly reviewed the literature (Camus 1936–

1954, Soepadmo 1972, Ho 1999, Huang et al. 1999, Ban 2005, Phengklai 2008) related to Quercus in Vietnam and surrounding countries. Based on the cupule morphology, Quercus trungkhanhensis is considered to be a member of subgenus Quercus (scale-cup oaks; Nixon 1993). According to the key in the Flora of China (Huang et al. 1999), Q. trungkhanhensis corresponds to Q. engleriana Seemen or Q. marlipoensis Hu & Cheng in the persistent leathery leaves with acute apex, cupule bracts scale-like, petiole 1-3 cm long, mature leaf blade abaxially glabrous or early glabrescent. According the acorn-based key in the Flora of Thailand (Phengklai 2008), Q. trungkhanhensis corresponds to Q. franchetii Skan in scaly cupule wall, cup-shaped cupule, each infructescence with (1 or)2 acorns, and serrate leaf margin. We therefore compared Q. trungkhanhensis with Q. engleriana, Q. marlipoensis and O. franchetii. For morphological comparison, we examined specimens using the websites of JSTOR Global Plants (http://plants.jstor.org) and the Chinese Virtual Herbarium (hereafter CVH: http://www.cvh.org.cn/). We also examined more than two hundred dried specimens kept in the herbaria DLU, FU, HN, P and VNM.

DNA barcoding

For DNA isolation, a piece of leaf was collected and desiccated using silica gel in the field. DNA was isolated by the CTAB method (Doyle & Doyle 1987) with minor modifications described in Toyama *et al.* (2015). We determined sequences of two DNA barcode regions; the internal transcribed spacer (ITS) and the large subunit of maturase K (*matK*) using the published protocols of Rohwer *et al.* (2009) and Dunning and Savolainen (2010) with a minor modification using Tks GflexTM DNA Polymerase (TAKARA, Japan) in the PCR amplification.

Phylogenetic analysis

We constructed a phylogenetic tree using nucleotide sequences of the DNA barcoding regions of ITS (487 bp) for 9 species including 8 species of *Quercus* and one species of *Lithocarpus* (Table

2). In addition to *Quercus trungkhanhensis*, we included *Q. engleriana*, *Q. franchetii* and three other species of subgenus *Quercus* for which ITS sequences were available in GenBank. No ITS sequence of *Q. marlipoensis* was available in GenBank. Two species of *Quercus* subgenus *Cyclobalanopsis* and *Lithocarpus dahuoaiensis* Ngoc & L. V. Dung were used as outgroups. Sequence alignment was performed by ClustalW with default parameter implemented in MEGA v.7.0 (Kumar *et al.* 2016).

The Neighbor-joining method (Saitou & Nei 1987) with Maximum Composite Likelihood distance matrix (Tamura *et al.* 2004) implemented in MEGA v.7.0 was used to construct the phylogenic tree. Confidence values for individual branches were determined by bootstrap analysis with 10,000 times resampling of the data.

Results

The comparison of characters among *Qercus* trungkhanhensis, Q. engleriana and Q. franchetii is shown in Table 1. Among the four syntypes of O. engleriana available on the webpage of JS-TOR Global Plants (A. Henry 5682, 1885-1888, China, BM, US and two specimens at GH), A. Henry 5682A (GH) is the only fruiting specimen. *Ouercus trungkhanhensis* is distinct from *Henry* 5682A in the size and morphology of the mature fruits. The mature fruits of A. Henry 5682A (Q. engleriana) are 12.5-13 mm long, with cupules 3.8–5.5 mm tall and 8.5–10 mm wide, acorns 5–7 mm long above the cupule and 6.5–7.5 mm wide, and the stylopodium is ca. 2 mm long. In Q. trungkhanhensis the mature fruits are 16-22 mm long, with cupules 8–10 mm tall and 12–14 mm wide, acorns 8-12 mm long above the cupule and 10–12 mm wide, and the stylopodium is ca. 2 mm long. The acorns are ovoid and acute at apex in Q. engleriana, but cylindrical and slightly concave at apex in Q. trungkhanhensis. The cupules are cup-shaped and relatively loosely covered with scales on the lower half in Q. engleriana but more cylindrical and tightly covered with scales in Q. trungkhanhensis (Fig. 2D-F). There are many

images of O. engleriana in CVH in showing the above distinctions to be mostly stable. As for fruit morphology of O. engleriana, PE00297544 [Nanchuan, Chongqing, alt. 1750 m, 6 Oct. 1957, J.-H. *Xiong & Z.-L. Zhou 93826* (PE): http://www.cvh. org.cn/spm/PE/00297544] has exceptionally more cylindrical acorns but the acorns are less than 10 mm long, as in typical O. engleriana. In A. Henry 5682A, the fruiting branchlets have 12 terminal or lateral buds that are narrowly ovoid, 5–9 mm long and 3–6 mm wide, whereas in Q. trungkhanhensis the buds are broadly ovoid, 3-4 mm long and 2-3 mm wide (Fig. 2A, Fig. 3B). The shape of the narrowly ovoid buds is stable among specimens of Q. engleriana in CVH. The young branchlets of Q. trungkhanhensis are appressed hairy with yellowish brown hairs (Fig. 2A), but the branchlets of *Q. engleriana* are yellowish gray tomentose. Quercus trungkhanhensis is also distinct from O. engleriana in having nearly glabrous leaves on fruiting branchlets; Q. engleriana usually retains dense hairs along the abaxial veins and on the petioles. According to the images on the CVH website, hairiness of the leaves of fruiting branchlets is somewhat variable, but O. engleriana retains dense hairs at least in the vein axils.

The digital images of the holotype (PE00039496) of Quercus marlipoensis available on the CVH webpage shows much larger leaves with more lateral veins (leaf blade 12-22 × 6-11 cm, with 11-16 pairs of lateral veins) than Q. trungkhanhensis (leaf blade $9-15 \times 2-5$ cm, with 8-10 pairs of lateral veins) and Q. engleriana. Among four specimens of Quercus marlipoensis at PE, PE00022946 (http://www.cvh.org.cn/spm/ PE/00022946) has three cupules which are 13-15 mm wide and larger than those of Q. engleriana (8.5–10 mm wide) and as large as Q. trungkhanhensis (12-14 mm wide). In PE00022946 the buds are narrowly ovoid (12×7 mm) as in Q. engleriana. In all four specimens the leaves and petioles are almost glabrous as in Quercus trungkhanhensis and the branchlets are less hairy than in Quercus trungkhanhensis and O. engleriana.

Six images of syntypes of *Quercus franchetii*

TABLE 1. Morphological comparison between Quercus trungkhanhensis Binh & Ngoc, sp. nov. with Quercus engleriana Seemen, Quercus franchetii Skan and Q. marlipoensis Hu & Cheng. Descriptions of fruit characters are based on mature

| Characters | Q. trungkhanhensis | Q. engleriana | Q. marlipoensis | Q. franchetii |
|---------------------------|---|--|--|---|
| Buds shape | Broadly ovoid | Narrowly ovoid ⁽¹⁾ | Narrowly ovoid | Narrowly ovoid ⁽⁴⁾ |
| Twigs | Yellowish brown appressed stellate hairy | Yellowish gray tomentose ⁽²⁾ | Yellowish brown tomentose ⁽²⁾ | Yellowish gray simple and fascicled hairs ⁽²⁾ |
| Leaf margin | Serrate upper (4/5–)2/3 | Serrate upper 1/2, sometime entire ⁽²⁾ | Scattered teeth or entire and slightly inflexed ⁽²⁾ | Serrate upper 1/2, setaceous at ends of teeth ^{(2),(3)} |
| Leaf surface | Glabrous on both surfaces except midrib and lateral veins | Densely yellowish brown pubescent ⁽²⁾ | Abaxially stellate tomentose along midvein ⁽²⁾ | Glabrous on the upper surface; densely yellowish gray tomen- tose below in the lower ⁽⁴⁾ |
| Leaf base | Rounded or shallowly cordate | Rounded, broadly cuneate, or rarely shallowly cordate ⁽²⁾ | Rounded ⁽²⁾ | Cuneate to cordate or obtuse ^{(2),(3)} |
| Leaf size | (7–)9–12.5 × 2.4–5.8 cm | $6-16 \times 2.5-5.5 \text{ cm}^{(2)}$ | $12-22 \times 6-11 \text{ cm}^{(2)}$ | $5-12 \times 2.5-6 \text{ cm}^{(2)}$ |
| Length of petioles | 1.8-2.4 cm long | 1–2 cm long ⁽²⁾ | 1.5–3 cm ⁽²⁾ | 0.2-1.4 cm long ^{(3), (4)} |
| Number of secondary veins | 8–10 pairs | 10–13 pairs ⁽²⁾ | 16–20 pairs ⁽²⁾ | 6–9 pairs ^{(3), (4)} |
| Cupule size | 8–10 mm tall, 12–14 mm in diam. | 3.8–5.5 mm tall, 8.5–10 mm in diam. ⁽¹⁾ | 8 mm tall, 14 mm in diam. (2) | (4–)7–12 mm tall, 10–14 mm in diam. ⁽²⁾ |
| Scales of cupule | Triangular | Ovate-lanceolate ⁽¹⁾ | Ovate ⁽²⁾ | Triangular ⁽²⁾ |
| Nut enclosure | Enclosing 1/3–1/2 of the nut | Enclosing 1/3–1/2 of the nut ⁽²⁾ | N/A | Enclosing 1/2 of the nut ⁽²⁾ |
| Apex of nut | Truncate and slightly concave | Acute ⁽¹⁾ | N/A | Slightly concave ^{(3), (4)} |
| Infructescence | 1–1.5 cm long, each infructescence with (1 or)2 acorns | 1–5 cm long, each infructescence with 1–10 acorns ⁽²⁾ | N/A | 1–5 cm long, each infructescence with (1 or)2 acorns ⁽³⁾ |

⁽¹⁾ From the material A. Henry 5682 (GH)

are on the webpage of JSTOR Global Plants [Henry A 9298, Yunnan, China, A(2), K(2), NY, US]. Quercus trungkhanhensis is easily distinguished from O. franchetii by having nearly glabrous leaves on the fruiting branchlets (vs. densely yellowish gray tomentose on the lower surface). Among the six syntypes, two specimens (A and K) have mature fruits have cup-shaped, somewhat cylindrical cupules 8-9 mm and tightly covered with scales and cylindrical acorns 10-

12 mm long and 8-9 mm wide and slightly concave at the apex.

The Neighbor-joining tree base on ITS (Fig. 4) showed that *Quercus trungkhanhensis* is sister to Q. franchetii with 67% bootstrap probability and O. engleriana is sister to those two species with 77% bootstrap probability. Quercus trungkhanhensis differed from Q. franchetii in four nucleotides and from *Q. engleriana* in ten nucleotides.

⁽²⁾ From the description in flora of China (Huang *et al.* 1999) (3) From the description in flora of Thailand (Phengklai 2008)

⁽⁴⁾ From the material A. Henry 9298 (K)

| Subgenus | Species | GenBank accession no |
|---|---|----------------------|
| | Quercus acutissima Carruth. | AF098428 |
| | Quercus engleriana Seemen | AY040465 |
| Subg. Quercus | Quercus franchetii Skan | AY040464 |
| | Quercus griffithii Hook. f. & Thomson ex Miq. | AY040490 |
| | Quercus variabilis Blume | AY040463 |
| | Quercus myrsinifolia Blume | AF098414 |
| Subg. Cyclobalanopsis | Quercus lamellosa Sm. | AY040454 |
| Outgroup Lithocarpus dahuoaiensis Ngoc & L. V. Dung | | KY436002 |

TABLE 2. List of taxa used in this study with GenBank accession number for the sequence of ITS region.

Taxonomy

Quercus trungkhanhensis Binh & Ngoc, sp. nov. —Figs. 2–3.

Quercus trungkhanhensis is morphologically similar to Q. engleriana and Q. marlipoensis, both distributed in China, in having persistent leaves, acuminate leaf apex, cupules covered with scales, petiole 1–3 cm long, mature leaf blades abaxially glabrous or early glabrescent, and leaf blade leathery; Q. trungkhanhensis differs from Q. marlipoensis in having smaller leaves, and differs from Q. engleriana in having larger cupule, nuts cylindrical slightly concave at apex (vs. nuts ovoid and acute at the apex), larger cupules tightly covered with scales (vs. relatively sparsely covered with scales on basal half), broadly ovoid buds (vs. narrowly ovoid), and yellowish brown appressed hairy branchlets when young (vs. yellowish gray tomentose).

Typus. VIETNAM. Cao Bang Province, Trung Khanh District, limestone subtropical evergreen mixed forests of Cao Vit Gibbon Conservation Area, 22°54'55"N, 106°31'28"E, alt. 767 m, 6 Nov. 2016, *Binh HT*, *Ngoc NV*, *Tai VA*, *Son HT V6066* (holo- KYO!, iso- DLU!, FU!).

Description. Tree, 5–10 m tall, 15 cm in girth. Bark pale gray, deeply longitudinally furrowed. Buds broadly ovoid, ca. 3–4 mm long, ca. 2–3 mm in diam.; scales in 4–6 rows, imbricate, ovate-triangular, ca. 3×2.5 mm, apex obtuse, margin ciliate, appressed hairy on both surfaces. Twigs densely yellowish brown stellate hairy when young, later glabrescent, lenticellate.

Leaves alternate; petiole 1.8–2.4 cm long, densely yellowish brown hairy when young, glabrescent later; blade leathery, ovate or ovate-elliptic, $(7-)9-12.5 \times 2.4-5.8$ cm, base rounded or shallowly cordate, margin serrate in upper (4/5–)2/3, apex acuminate, pale brown or reddish brown when dry, both surfaces glabrous except stellate hairs remaining near base of midrib on upper surface and in axils of secondary veins on lower surface; midrib slightly raised on upper surface, prominently raised on lower surface; lateral veins 8-10 pairs, prominent, at angle of 50-60(-70) degree from midrib, straight and running into marginal teeth, tertiary veins scalariform, faintly visible. Inflorescences (staminate and carpellate) not seen. Infructescences axillary or terminal, erect; peduncle woody, ca. 1 cm long; rachis 1-1.5 cm long, 4-5 mm in diam., glabrous, brownish red when fresh, blackish brown when dried, lenticellate. Mature fruits 1.6–2.2 cm long (including cupule), solitary or paired, sessile; acorns ovoid, 1-1.5 cm long, 1-1.2 cm in diam., truncate and slightly concave at apex, white tomentose when young, densely appressed hairy around stylopodium, stylopodium to 2 mm long, basal scar 5-6 mm in diam., raised; cupules cup-shaped, somewhat cylindrical, 0.8-1 cm tall, 1.2-1.4 cm in diam., enclosing 1/3 to 1/2 of the mature acorn, scales on cupule triangular, ca. 1-1.5 mm long on lower part of cupule, smaller on upper part, apex short acuminate or rarely acute, dull greenish yel-

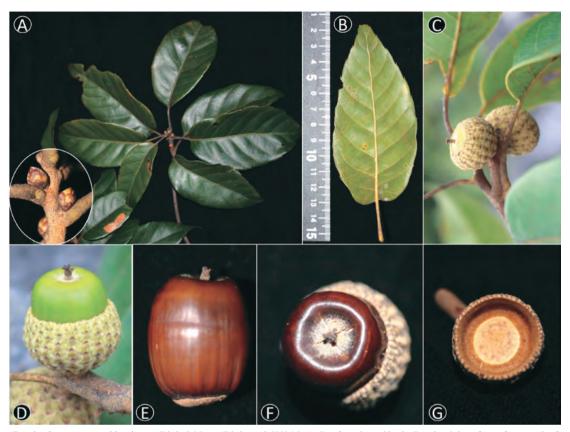


Fig. 2. Quercus trungkhanhensis Binh & Ngoc (Binh et al. V6066). A. Leafy twig and buds, B. Abaxial surface of mature leaf, C. Infructescence and young fruits, D. Mature fruit, E. Nut (lateral view), F. Nut (top view), G. Inside of cupule.

low, dark purplish red near apex, densely appressed hairy with short grayish brown hairs outside, densely hairy with short yellowish brown hairs inside.

Phenology. Fruiting specimens were collected in January.

Distribution and habitat. Known only from Cao Vit Gibbon Conservation Area, Trung Khanh District, Cao Bang province, Vietnam (Fig. 1). We found only two individuals within 100 m² on a ridge in a limestone subtropical evergreen mixed forest, at 767 m altitude.

Etymology. The specific epithet is derived from the district name of the type locality, Trung Khanh District, Cao Bang Province, northeastern Vietnam.

GenBank accession no. Binh et al. V6066: KY867547 (ITS), LC258443 (matK).

Conservation status. Critically endangered (CR). Quercus trungkhanhensis is known only from two individuals. The forest in the Conservation Area is currently protected under law from anthropogenic activities but the locality of Q. trungkhanhensis was disturbed by local people searching for forest resources. Although additional individuals/populations of Q. trungkhanhensis may be discovered, it qualifies as CR under criterion B in that the area of occupancy is less than 10 km² at only a single location and criterion D of the population size is fewer than 50 mature individuals (IUCN 2001).

Note. Quercus trungkhanhensis is a member of Quercus subgenus Quercus (scale-cup oaks;

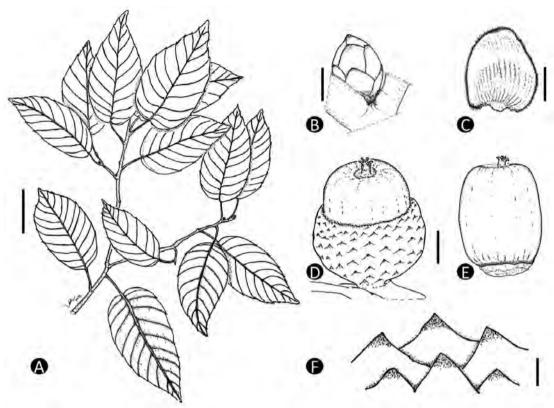


FIG. 3. Line drawing of *Quercus trungkhanhensis* Binh & Ngoc (*Binh et al. V6066*). A. Leafy twig, B. Bud, C. Bud scale, D. Mature fruit, E. Nut, F. Cupule scales. Scale bars A = 5 cm, B = 2 mm, C = 1 mm, D & E = 5 mm, F = 1 mm.

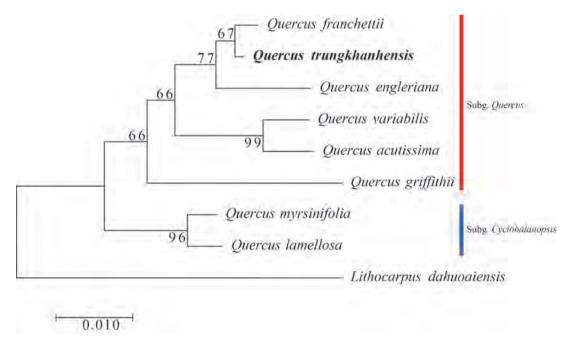


FIG.4. NJ tree of *Quercus trungkhanhensis* and seven species of subgenus *Quercus* and subgenus *Cyclobalanopsis* based on data from nuclear ribosomal ITS region. Branches are labeled with bootstrap support (% of 10,000 replicates)

Nixon 1993), corresponding to *Ouercus* s. str., excluding Cyclobalanopsis (Huang et al. 1999). In Thailand (Phengklai 2008), most species of Quercus belong to subgenus Cyclobalanopsis (cyclecup oaks; Nixon 1993). Phengklai (2008) listed only seven species of subgenus Quercus. Similarly, in Vietnam there are fewer species of subgenus Quercus than of subgenus Cyclobalanopsis. Quercus trungkhanhensis is easily distinguished from the eight other species of subgenus Ouercus (O. acutissima Carruth., O. aliena Blume, O. franchetii, O. kingiana Craib, O. lanata Sm., O. oblongata D. Don, O. setulosa Hickel & A. Camus and *Q. variabilis* Blume) in the leathery mature leaves abaxially glabrous or early glabrescent.

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